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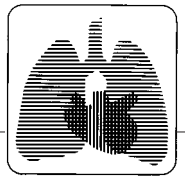
Chest 1998;113;505-513



occupational and environmental lung disease

The Effect of Glucocorticoids on Grain Dust-Induced Airway Disease*

John F. Trapp, MD; Janet L. Watt, BA; Kathy L. Frees; Timothy J. Quinn, BS; Matthew W. Nonnenmann; and David A. Schwartz, MD, MPH, FCCP

Study objectives: **To determine the effect of glucocorticoids on grain dust-induced airflow obstruction and airway inflammation.**
Design: **Randomized controlled trial.**
Setting: **University hospital.**
Participants: **Health volunteers.**
Interventions: **Two randomized, placebo-controlled trials, each studying 10 healthy volunteers who were pretreated with either triamcinolone acetonide (Azmacort) oral inhaler 4 puffs twice daily (800 μg daily) for 7 consecutive days or IV hydrocortisone (3 μg/kg/min) as a 14-h continuous infusion, then subjected to a controlled inhalation exposure to corn dust extract (CDE) (endotoxin exposure dose of 3 μg/kg). A single-blind, crossover study design was performed for each trial enrolling 10 healthy, lifetime nonsmokers, with no history of lung disease or environmental exposure to grain dust.**
Measurements and results: **Following each inhalation exposure to CDE, spirometry was performed at regular intervals and BAL was performed at 4 h. Both treatment and placebo groups demonstrated significant decrements in spirometry and increments in BAL cellularity following CDE inhalation compared with placebo. Inhaled steroid treatment resulted in a significantly higher FEV_1 only at the 2-h time point following CDE inhalation with no significant differences observed in the BAL total cell concentration or cellular differential compared with placebo. IV hydrocortisone treatment resulted in a significantly higher FEV_1 and FVC between 2 and 4 h after CDE inhalation, as well as significant reductions in the BAL total cell, macrophage, and eosinophil concentrations. Interestingly, the concentration of tumor necrosis factor-α and interleukin-8 in the BAL fluid was also decreased following treatment with IV glucocorticoids.**
Conclusions: **These results demonstrate that glucocorticoids, administered IV and perhaps by inhalation, have a mildly protective effect on airflow obstruction and airway inflammation induced by inhalation of grain dust.** ***(CHEST 1998; 113:505-13)***

Key words: airway injury; asthma; glucocorticoids; grain dust

Abbreviations: BPI=bactericidal permeability-increasing protein; CDE=corn dust extract; GCRC=General Clinical Research Center; HC=hydrocortisone; IL=interleukin; LPS=lipopolysaccharide; MDI=metered-dose inhaler; MIP=macrophage inhibitory protein; $rBPI_{23}$=recombinant BPI; SS=sterile saline (solution); TNF=tumor necrosis factor

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This study was supported by grants from the National Institute of Environmental Health Sciences (ES06537, ES07498, ES05605) and the General Clinical Research Centers Program (RR00059).
Manuscript received June 25, 1997; revision accepted August 25.
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Occupational exposure to grain dust can cause a broad range of respiratory disorders, including grain fever, asthma, bronchitis, acute and chronic changes in airway reactivity, and progressive irreversible airflow obstruction.[1] In North America, it is estimated that >5 million agricultural workers are exposed to grain dust each year.[1-4] Among grain handlers, the prevalence of work-shift changes in FEV_1 (>10% decline) varies between 3.9% and 11%.[2-4] The prevalence of chronic bronchitis in

nonsmoking grain handlers has been estimated to be as high as 23 to 37%.[5-8] Epidemiologic studies have suggested that these acute decrements in airflow across a work shift or work week are consistently associated with longitudinal declines in lung function.[9-11] Therefore, we think that a better characterization of the initial biological and physiologic mechanisms associated with short-term grain dust exposure is important for developing interventions that may lead to the treatment and prevention of grain dust-induced lung disease.

The acute inflammatory events following grain dust inhalation induce a neutrophilic response in the upper and lower airways that appears to be mediated through nonallergic mechanisms. Using a model of short-term grain dust inhalation, we have shown that healthy, nonatopic volunteers with no prior exposure to grain dust develop respiratory symptoms and significant declines in FEV_1 following grain dust exposure.[12] Additionally, these subjects develop a neutrophilic alveolitis associated with increases in the concentration of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 in the BAL fluid.[13]

Although the pathogenesis of grain dust-induced airway disease has not been clearly defined, growing evidence suggests that endotoxin may be the principal component of grain dust responsible for the development of airway inflammation and airflow obstruction. Endotoxin has been measured in similar concentrations in several vegetable dusts, including corn, wheat, oats, barley, and cotton.[14] The concentration of inhaled endotoxin is strongly associated with the development of airflow obstruction among agricultural workers, including grain workers,[15] swine confinement workers,[16] poultry workers,[17] and those exposed to cotton dust.[18-20] Moreover, the concentration of endotoxin in the bioaerosol is clearly associated with progressive airflow obstruction in agricultural workers.[15]

Inhalation exposure of healthy volunteers to grain dust and endotoxin has demonstrated similar clinical symptoms, decrements in airflow, and increases in BAL inflammatory cells and mediators.[13,21-23] Reductions in the concentration of endotoxin in grain dust result in a substantial decline in grain dust-induced airflow obstruction and airway inflammation.[24] Endotoxin responsiveness appears to be required for the development of the inflammatory response following inhalation of grain dust as demonstrated in acquired and genetic models of endotoxin hyporesponsiveness.[25,26] Competitive inhibition of endotoxin also results in a dose-dependent reduction in grain dust-induced inflammation.[27]

Given the complexity and incomplete characterization of the inflammatory response following grain dust inhalation and the finding that endotoxin appears to be driving the inflammatory response, we considered altering the inflammatory response using an agent that is well described to inhibit airflow obstruction and airway inflammation in asthma and appears to be active in endotoxin-mediated inflammation. Glucocorticoids, and in particular, inhaled glucocorticoids, have become the mainstay of therapy for managing the inflammatory response in asthma. Glucocorticoids appear to produce their effect on responsive epithelial airway cells through the activation of glucocorticoid receptors and by direct and indirect interactions with transcription factors, such as AP-1 and nuclear factor-kappa B, which are important in regulating cytokine expression in the airway inflammatory response.[28,29] In a rat model using intratracheal coadministration of lipopolysaccharide (LPS) and dexamethasone, glucocorticoids significantly reduced concentrations of TNF-α, IL-1β, IL-6, macrophage inhibitory protein (MIP)-1α, MIP-2, and macrophage chemoattractant protein-1, resulting in inhibition of both the vascular and cellular aspects of LPS-mediated inflammation.[30]

Therefore, the purpose of this present investigation is to determine whether inhaled and IV glucocorticoids administered prior to inhalation of grain dust alter the physiologic and inflammatory events in healthy volunteers. We hypothesize that pretreatment of subjects with glucocorticoids prior to grain dust inhalation will reduce grain dust-induced airflow obstruction and the inflammatory response in the lower respiratory tract.

Materials and Methods

We used a single-blind, crossover study design to determine whether pretreatment with inhaled steroid or IV hydrocortisone reduces the physiologic and inflammatory response to inhalation of corn dust extract (CDE) in healthy volunteers.

Study Subjects

Ten healthy, nonatopic, nonasthmatic, never-smoking subjects without any history of cardiopulmonary disease or occupational exposure to grain dust were recruited to participate in each of the placebo-controlled trials. The study protocols were approved by our human subjects review committee and carried out at the University of Iowa-National Institutes of Health General Clinical Research Center. To be considered eligible for participation, all study subjects were required to have normal results of a physical examination, 12-lead ECG, chest radiograph, negative urine β-human chorionic gonadotropin (if indicated), and pulmonary function tests, including spirometry, lung volumes, and diffusing capacity. Skin testing for 10 common aeroallergens was performed in all subjects to determine atopic status. An abbreviated histamine challenge test was performed on each subject.[31] Subjects demonstrating a 20% or greater reduction in their baseline FEV_1 compared with diluent were excluded from study partici-

pation. Using this protocol, a negative histamine challenge corresponds to a provocative concentration of substance causing 20% fall in FEV_1 of >16 mg/mL or provocative concentration >85 cumulative breath units of histamine.

Protocol

Study subjects underwent two separate inhalation challenges, with each exposure being separated by at least 2 weeks. Based on previous kinetic studies of grain dust performed in our laboratory, this interval of time is sufficient for lung function and lavage parameters to return to baseline values.[32]

Inhaled Steroid: Study subjects were randomized to receive inhaled steroid or placebo metered-dose inhaler (MDI) prior to the first inhalation challenge. Subjects were instructed to use four oral puffs twice daily for 7 days prior to the challenge. Compliance was assessed by direct phone contact through the General Clinical Research Center (GCRC) at the expected time of each scheduled dose to verify administration or to remind the subject to take the scheduled dose. On the day of the exposure, subjects received four puffs of their inhaler 2 h prior to inhalation challenge. Vital signs and pulmonary function testing were measured prior to and following completion of the inhalation challenge. Bronchoscopy was then performed approximately 4 h following completion of the inhalation challenge. For the second exposure, subjects received the intervention they had not received during the first exposure, following the same dosing schedule and markers of compliance. Study subjects were blinded to the treatment intervention. Triamcinolone acetonide (Azmacort Oral Inhaler; Rhone-Poulenc Rorer Pharmaceuticals Inc; Collegeville, Pa) is a metered-dose aerosol unit containing a microcrystalline suspension of triamcinolone acetonide in the propellant dichlorodifluoromethane and dehydrated alcohol USP 1%. Each actuation releases approximately 200 μg of triamcinolone acetonide, of which approximately 100 μg is delivered from the unit. Placebo MDI (Allen and Hanburys Pharmaceuticals; Division of Glaxo Inc; Research Triangle Park, NC) was designed as a placebo unit delivering only aerosol propellant.

IV Hydrocortisone: Study subjects were randomized to receive hydrocortisone (HC) or sterile saline solution (SS) infusion prior to the first inhalation challenge. Subjects were admitted to the GCRC the evening prior to exposure where vital signs and nursing assessments were performed, and a 20-gauge Silastic IV catheter was placed. At midnight (time 0 h), a 14-h continuous IV infusion of HC (3 μg/kg/min) or equivalent volume of SS was initiated. Vital signs and nursing assessments were recorded at the start of the infusion, at 15, 30, 45, and 60 min, and on awakening at 7 AM the next morning. The inhalation challenge was performed at 9 AM. Vital signs and pulmonary function testing were measured prior to and following completion of the inhalation challenge. Bronchoscopy was performed immediately following completion of the infusion, at 2 PM, which was also approximately 4 h following the inhalation challenge. For the second exposure, subjects received the intervention they had not received during the first exposure, following the same admission process and dosing schedule as in the first exposure. Study subjects were blinded to the treatment intervention. Preparation of the infusion was performed in the GCRC by the nurse administering the infusion. The infusion was labeled as study drug and prepared by mixing HC, 250 mg in a 500-mL bag of SS or placebo as a 500-mL bag of SS. Dosage was calculated to determine the rate of infusion of the study drug with infusion volumes and rates being equivalent for both exposures.

Preparation of the CDE: Corn dust used in this study was obtained from the air filtration system at an eastern Iowa grain facility. CDE was prepared by mixing 3.0 g of dust in 30 mL (0.1% solution) of sterile, pyrogen-free Hanks' balanced salt solution without calcium or magnesium, vortexing for 2 min, and shaking for 1 h at 4°C. The mixture was centrifuged at 800×*g* for 20 min, and the supernatant solution was collected, resulting in CDE. The CDE solution underwent filter sterilization through a 0.22-μm filter (No. 09-730-256 Acrocap Low Protein Binding Filter Unit; Gelman Science; Ann Arbor, Mich). All solutions used for inhalation were derived from a stock solution, which underwent sterility testing (bacterial and fungal) and endotoxin assay prior to separation into individual aliquots. These aliquots were stored at −70°C until being used. Endotoxin concentration was measured by the end point chromogenic *Limulus* amebocyte lysate assay (QCL-1000; Whittaker Bioproducts; Walkersville, Md). The measured endotoxin concentration in the prepared CDE was 2.7 μg/mL for the inhaled steroid study and 2.4 μg/mL for the IV HC study.

Inhalation Challenge: The solutions were administered via a nebulizer (DeVilbiss 646) and dosimeter (DeVilbiss; DeVilbiss Health Care Inc; Somerset, Pa), operated at 20 psi air pressure. Subjects manually controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhale through their nose. Using this delivery system and technique, a precise dose of inhalant was delivered, obtaining maximal airway mucosal exposure. For each exposure, subjects were administered 0.1 mL of inhalant per kilogram of body weight, given over a 60-min period.

Pulmonary Function Testing: The pulmonary function tests consisted of serial spirometry using a spirometer (Spirotech S-600; Graseby Anderson; Atlanta). These maneuvers were performed using the standard protocol of the American Thoracic Society.[33] The spirometer was calibrated prior to each daily use. Spirometry was performed with noseclips while the subject was in a sitting position. Recordings were obtained immediately preexposure, and at the following postexposure time points: 10, 20, and 30 min, and 1, 2, 3, 4, and 24 h.

Bronchoscopy: Bronchoscopy was performed 4 h following each inhalation exposure, in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatics.[34] A fiberoptic bronchoscope (Olympus P-10; 1.5-mm channel; Olympus; Lombard, Ill) was introduced into the selected lung segment for lavage and placed in a wedge position. Twenty milliliters of normal SS (37°C) was injected through the bronchoscope and then collected. This was performed five additional times for a total lavage volume of 120 mL. The return of the first 20-mL aliquot was separated from the remaining lavage fluid. Lung segments selected for BAL alternated between a subsegment of the right middle lobe following the first exposure and a subsegment of the lingula following the second exposure.

Processing of the Specimens: Immediately following bronchoscopy, the BAL samples were processed according to methods described previously.[22] The BAL supernatant was frozen at −70°C for subsequent use. After washing the cells twice with Hanks' balanced salt solution, the cell pellet was suspended in RPMI-1640 medium and cell counts were performed. Cytospin preparations were made from the lavage cell resuspension, stained with a Giemsa-type stain (Diff-Quik; Baxter Scientific Products; Miami, Fla), and cell differential counts were quantified counting 200 cells. TNF-α and IL-8 were measured in the BAL supernatant fluid using commercially available immunoassay kits (R&D Systems Inc; Minneapolis).

Statistics

CDE-induced physiologic changes in lung function (spirometry) and biological measures of inflammation (BAL cellularity) following treatment with glucocorticoids were compared with the CDE-induced responses following treatment with placebo. Analysis of numeric data was performed using paired, one-tailed,

nonparametric statistics (Wilcoxon Signed-Rank Test). χ^2 analysis was performed in comparing symptom frequencies following treatment with glucocorticoids vs placebo.

Results

Inhaled Steroids

In the inhaled steroid study group, 10 subjects (5 male, 5 female; mean age, 32 years; range, 21 to 41 years) participated in the study. Subject compliance of medication administration was verified through direct phone contact at the time of each scheduled treatment dose with 278 of 280 treatments received (99.3%). There were no significant adverse effects reported with the use of either inhaled steroid (sore throat, one; sinus congestion, two) or placebo MDI (sore throat, two; nasal congestion, two; headache, two; dyspepsia, one). Acute respiratory symptoms were recorded following each inhalation exposure to CDE and included chest tightness, cough, dyspnea, and sputum production. Other reported constitutional symptoms included chills, myalgias, sinus congestion, malaise, nausea, headache, and feeling cold. The frequency of these reported symptoms was not significantly different between the two treatment groups (Table 1).

Following inhalation challenge with CDE, significant reductions in the FEV_1 and FVC occurred as early as 10 min postexposure and persisted for at least 4 h. All spirometric values returned to baseline by 24 h. Prechallenge spirometric values for each individual were not significantly different between the two exposures. Comparing subjects pretreated with placebo MDI vs inhaled steroid, a significant difference in FEV_1 was measured at 2 h postexposure (Fig 1), with inhaled steroids resulting in a diminished decline in FEV_1. No other significant differences between placebo MDI-treated and inhaled steroid-treated subjects were observed in the FEV_1 or FVC at the other time points measured ($p>0.05$).

Inhalation challenge to CDE resulted in short-term increases in BAL total cell concentrations and neutrophil concentrations for both placebo MDI-pretreated and inhaled steroid-pretreated subjects. No significant differences were observed in the mean BAL total cell concentrations, neutrophil concentrations, macrophage concentrations, lymphocyte concentrations, or eosinophil concentrations between placebo MDI-pretreated and inhaled steroid-pretreated subjects, respectively ($p>0.05$) (Fig 2).

Inhalation challenge to CDE resulted in short-term increases in BAL cytokine levels at 4 h, including TNF-α and IL-8 for both placebo MDI-pretreated and inhaled steroid-pretreated subjects. However, no significant differences were observed between the BAL TNF-α levels or BAL IL-8 levels between the placebo MDI-pretreated and inhaled steroid-pretreated subjects, respectively ($p>0.05$) (Fig 3).

IV HC

For the HC study group, 10 subjects (5 male, 5 female; mean age, 33 years; range, 23 to 46 years) participated in the study. No adverse effects were reported during the infusion of HC or equivalent volume of SS. Again, acute respiratory symptoms were reported following each inhalation exposure to CDE and included chest tightness, cough, dyspnea, and sputum production. Other reported constitutional symptoms included chills, myalgias, sinus congestion, nausea, malaise, headache, and feeling cold.

Table 1—*Frequency of Symptoms Reported Following Inhalation Exposure to CDE

	Inhaled Steroid Trial		IV Steroid Trial		
Symptoms	Inhaled Steroid (n=10)	Placebo MDI (n=10)	HC (n=10)	Placebo (n=10)	p Value†
Chest tightness	7	6	5	7	NS
Cough	6	3	6	7	NS
Shortness of breath	5	2	4	2	NS
Sputum production	2	2	4	2	NS
Chills	3	5	1	2	NS
Myalgias	0	1	3	1	NS
Sinus congestion	4	2	2	4	NS
Nausea	1	3	4	3	NS
Malaise	3	2	1	2	NS
Headache	2	2	2	1	NS
Feeling cold	7	8	5	9	NS

*Values represent actual number of subjects reporting symptoms.
†NS=not significant (p value >0.05).

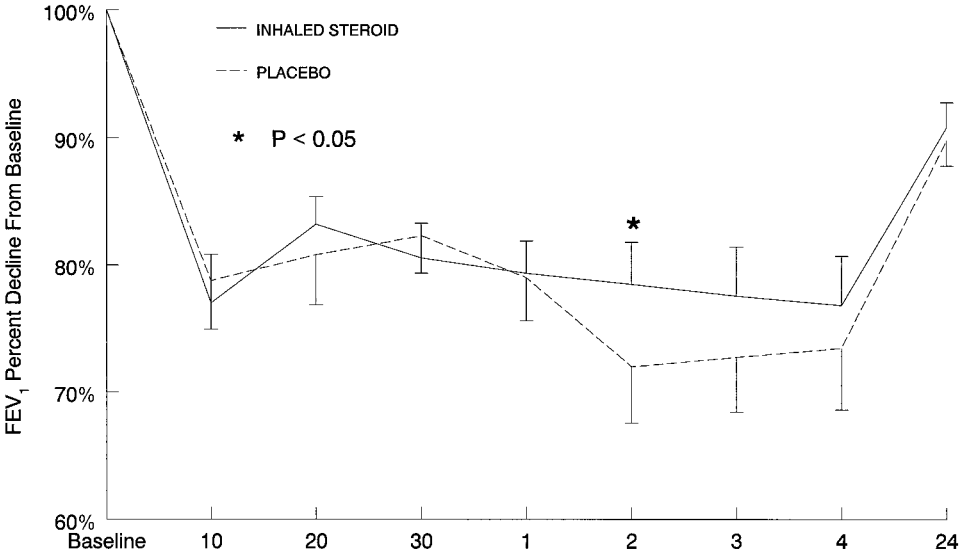

FIGURE 1. The mean FEV_1 for the inhaled steroid trial expressed as percent decline from baseline at times 10, 20, and 30 min and 1, 2, 3, 4, and 24 h after inhalation of CDE. Error bars represent the SE. Asterisk represents a p value <0.05.

The frequency of these reported symptoms was not significantly different between the two treatment groups (Table 1).

Following inhalation challenge with CDE, significant reductions in the FEV_1 and FVC occurred at 10 min postexposure and persisted for at least 4 h, returning to baseline at 24 h. Prechallenge spirometric values for each subject were not significantly different between the two exposures. However, a significant reduction in airflow obstruction was observed in the HC-treated group in both the FEV_1 (2 h, 4 h) (Fig 4) and in the FVC (3 h, 4 h). The percent of baseline FVC±SEM at 3 and 4 h for SS compared with HC was 82.2±4.2 vs 87.4±3.0 (p=0.05) and 81.6±4.1 vs 88.7±2.6 (p=0.01), respectively.

Inhalation challenge to CDE resulted in short-term increases in BAL total cell concentrations and neutrophil concentrations for both the SS and the HC pretreated subjects. Significant reductions were observed in the mean BAL total cell concentrations ($2.24\pm0.59\times10^6$ vs $1.30\pm0.29\times10^6$; p=0.04), BAL macrophage concentrations ($6.18\pm1.24\times10^5$ vs $3.30\pm0.66\times10^5$; p=0.02), and BAL eosinophil concentrations ($1.19\pm0.83\times10^4$ vs $0.28\pm0.03\times10^4$; p=0.03) between the SS- and HC-pretreated subjects, respectively. Although IV HC suppressed the recruitment of

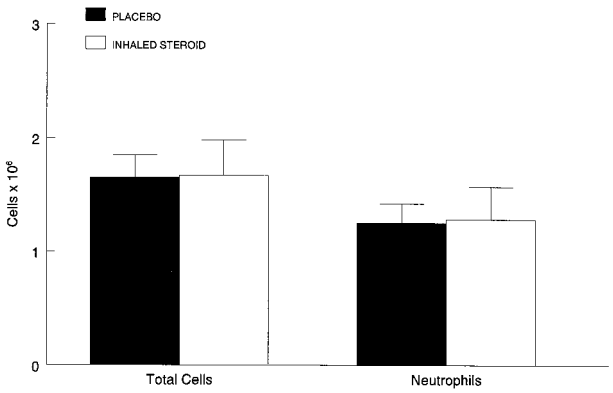

FIGURE 2. The mean concentrations of BAL total cells and neutrophils expressed as cells $\times10^6$ in the inhaled steroid trial following inhalation exposure to CDE. Error bars represent the SE.

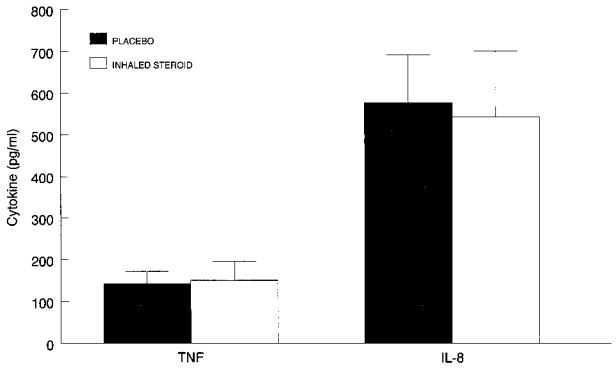

FIGURE 3. The mean concentrations of BAL TNF-α and IL-8 expressed as picograms per milliliter in the inhaled steroid trial following inhalation exposure to CDE. Error bars represent the SE.

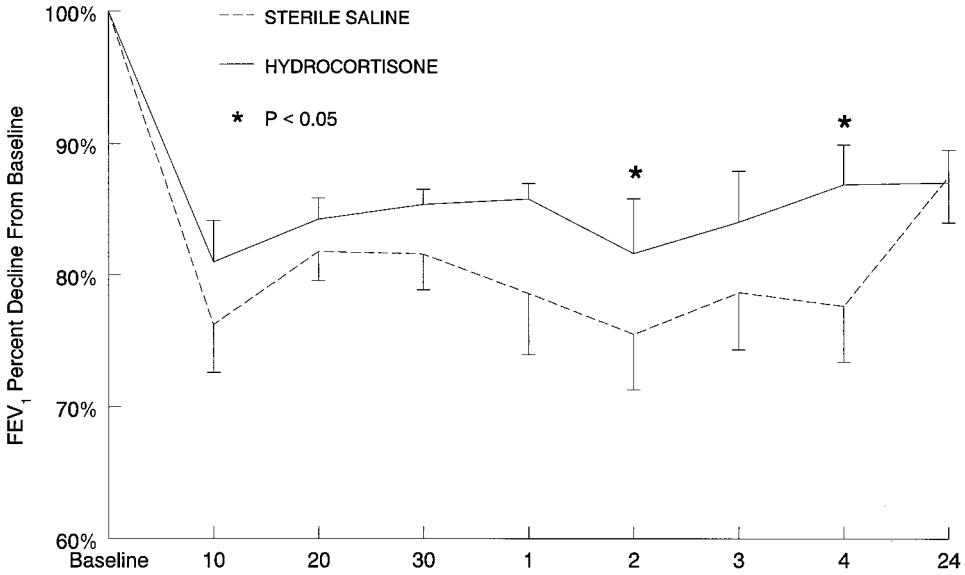

FIGURE 4. The mean FEV_1 for the IV steroid trial expressed as percent decline from baseline at times 10, 20, and 30 min and 1, 2, 3, 4, and 24 h after inhalation of CDE. Error bars represent the SE. Asterisks represent a p value <0.05.

neutrophils and lymphocytes into the airways, these differences were not statistically significant (Fig 5).

Inhalation challenge to CDE resulted in short-term increases in BAL cytokine levels at 4 h, including TNF-α and IL-8 for both the SS- and the HC-pretreated subjects. A significant reduction in cytokine levels was observed in the BAL TNF-α levels and BAL IL-8 levels between the SS- and HC-pretreated subjects, respectively (Fig 6).

DISCUSSION

Our results suggest that glucocorticoids, administered either by inhalation or by continuous infusion, have a mildly protective effect on airflow obstruction following inhalation of CDE. Furthermore, glucocorticoids administered by continuous infusion appear to reduce the BAL cellular concentration of macrophages, eosinophils, TNF-α, and IL-8, in grain dust-induced airway inflammation. However, no difference was observed in the concentration of BAL neutrophils. There did not appear to be any difference in respiratory or constitutional symptoms between subjects who received placebo and those who received glucocorticoids prior to CDE inhalation. Despite this slight protective effect of glucocorticoids, CDE still caused significant degrees of airflow obstruction and airway inflammation even following treatment with IV glucocorticoids.

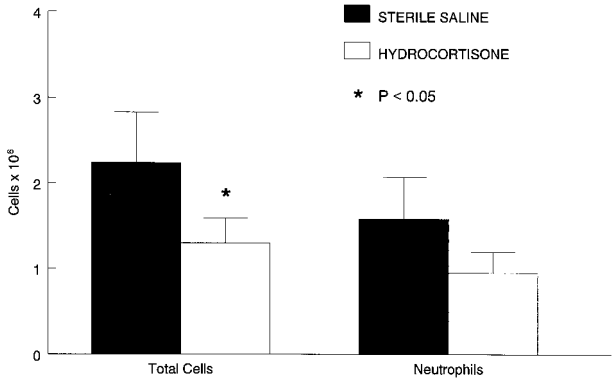

FIGURE 5. The mean concentrations of BAL total cells and neutrophils expressed as cells $\times 10^6$ in the IV steroid trial following inhalation exposure to CDE. Error bars represent the SE. Asterisk represents a p value <0.05.

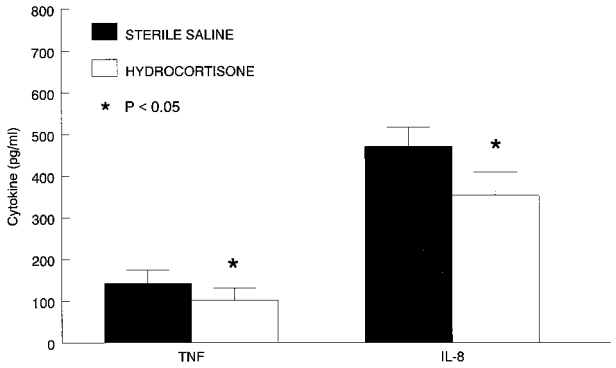

FIGURE 6. The mean concentrations of BAL TNF-α and IL-8 expressed as picograms per milliliter in the IV steroid trial following inhalation exposure to CDE. Asterisks represent p values <0.05. Error bars represent the SE.

These results were somewhat surprising since glucocorticoids at most resulted in a mildly protective effect on grain dust-induced airflow obstruction and airway inflammation. A potential explanation for our results presented in this study may be that there was an insufficient dose of glucocorticoids administered to subjects. The daily dose of inhaled steroids administered in this study was a standard dose used to treat airway inflammation in asthmatic patients. The duration of pretreatment was for a period of 7 days, which may have been insufficient to result in a significant reduction in airflow obstruction and airway inflammation. Additionally, the dose of four puffs twice daily with an expected delivered dose of 800 μg/d may have been insufficient in preventing the inflammatory response initiated by a short-term inhalation exposure of CDE. The continuous infusion of HC did indeed provide patients with a higher dose of glucocorticoids and in fact did result in a more pronounced protective effect; however, this remained a relatively mild effect on airflow obstruction and airway inflammation. Previous studies, using both higher concentrations of intratracheal LPS and doses of intraperitoneal steroid, have demonstrated a reduction in LPS-initiated acute pulmonary inflammation, including proinflammatory cytokines and cellular influx, in a dose-dependent manner using dexamethasone in a rat model of inflammation.[35] In our IV glucocorticoid-pretreated subjects, there was a significant, but relatively mild, attenuation of TNF-α and IL-8 levels, while there was no significant decrement seen in the inhaled steroid-pretreated subjects. These findings support the observation of O'Leary and Zuckerman[36] that there is a dose-dependent response between glucocorticoids and proinflammatory cytokine levels. In our study, as suggested by the minimal reduction in measured cytokine levels, there may have been an inadequate dose of glucocorticoids to significantly block the initial cascade of proinflammatory cytokines.

Another possibility is that CDE-induced inflammation is mediated through a pathway that is not blocked by glucocorticoids. As discussed earlier, there is a growing body of evidence that endotoxin is the principal component of grain dust responsible for the development of airflow obstruction and airway inflammation. In a recent trial using rats treated with dexamethasone (2 to 4 mg/kg) followed by intratracheal instillation of endotoxin (1 mg/kg), BAL analysis revealed a significant suppression of TNF-α levels and cellular influx into the alveolar space. However, measurements of BAL protein, which serves as an index of pulmonary microvascular leak, remained elevated and unaffected by steroid treatment. In this series of studies, MIP-2 level also remained elevated despite increasing doses of dexamethasone (2 to 40 mg/kg), suggesting that MIP-2 may be involved in an inflammatory pathway that is resistant to glucocorticoids.[35,36] Further evaluation of such a glucocorticoid-resistant pathway will need to be investigated.

Because of the clinical relevance in preventing endotoxin-related diseases, understanding the mechanism underlying endotoxin-mediated inflammation has become an area of intense research. Previous interventions using antiendotoxin immunotherapy in human trials have been investigated extensively.[37-44] Unfortunately, antiendotoxin antibodies have demonstrated only limited beneficial effect in reducing rates of morbidity and mortality in small subgroups of patients. This marginal therapeutic effect, along with the inability of the antibodies to neutralize endotoxin *in vitro*, has limited applications in using this therapy to treat human disease. Additional research has focused on LPS-binding proteins, including bactericidal permeability-increasing protein (BPI), *Limulus* anti-LPS factor, and LPS-binding protein. Small peptides derived from these binding proteins have demonstrated significant endotoxin-neutralizing and bactericidal activity *in vitro* against many Gram-negative bacteria.[45] BPI, which is a major constituent of the azurophilic granules on the surface of polymorphonuclear leukocytes, specifically interacts and has a high affinity for the lipid A portion of LPS. This protein, when bound to endotoxin, has been demonstrated to neutralize endotoxin activity *in vitro*[46] and *in vivo*.[47] Recombinant BPI ($rBPI_{23}$)[48] has been developed and has been shown to effectively bind to endotoxin[49] and neutralize many of the biological effects of endotoxin in human subjects.[50] Given the potent endotoxin-neutralizing capability of $rBPI_{23}$, further clinical use of $rBPI_{23}$ in the treatment of endotoxin-mediated disease is warranted. Cytokine regulation and signaling is another important area of research that may also substantially contribute to control of endotoxin-mediated diseases.

In conclusion, glucocorticoids, as administered by inhalation or IV routes with the doses used in this study, have only a mildly protective effect on airflow obstruction following CDE inhalation. This treatment, which has been proved to be relatively safe for the long-term management of asthma, is a suboptimal treatment in preventing grain workers from the physiologic and inflammatory events following short-term grain dust exposure. However, our current model of CDE-induced inflammation could be used to better characterize and develop interventions for endotoxin-mediated inflammation in human subjects. Certainly, further investigation of other novel agents capable of reducing the inflammatory response to grain dust and endotoxin is warranted in

order to better prevent and treat environmental and occupational lung disease associated with grain dust exposure.

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